



Optimization of Sweet Potato Starch Hydrolyzate Production and Its Potential Utilization as Substrate for Citric Acid Production

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Authors' contributions

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Research Article

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ABSTRACT

Aims: The aims of this work was optimization of two-step enzymatic hydrolysis of sweet potato starch using statistical approach and subsequent utilization of the hydrolyzate obtained for citric acid production.

Methodology: Box Behnken design was used in this study to generate a total of 17 individual experiments for each step of the hydrolysis (liquefaction and saccharification steps). These were designed to study the effect of temperature, time and pH on the sweet potato starch hydrolyzate (SPSH) concentration. The optimization was carried out using response surface methodology (RSM). The SPSH obtained was used to culture *Aspergillus niger* for citric acid production.

Results: A statistically significant quadratic regression model ($P < 0.05$) was obtained for the liquefaction step. Statistical model predicted the highest sweet potato starch hydrolyzate (SPSH) concentration to be 172.23 g/L at optimal condition of temperature 61.05°C, time 55.02 min and pH 6.5. A statistically significant quadratic regression model was also obtained for the saccharification step. Statistical model predicted the highest SPSH concentration to be 241.92 g/L, established at the optimal condition of temperature 52°C, time 44 min and pH 4.5. The optimal liquefaction and saccharification conditions were validated with the actual SPSH concentration of 172.00 and 241.01g/L, respectively. The maximum citric acid production of 86g/L was achieved on the 8th day of cultivation

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when the SPSH was used for the cultivation of *A. niger*.

Conclusion: RSM was successfully applied the two-step enzymatic hydrolysis of sweet potato starch. This work showed that the sweet potato starch hydrolyzate could serve as sole carbon source for citric acid production.

Keywords: Enzymes; optimization; response surface methodology; sweet potato; Aspergillus niger; cultivation; citric acid.

1. INTRODUCTION

Sweet potato (*Ipomoea batatas*) belongs to the *Convolvulaceae* (morning glory) family. It is a perennial plant that is widely cultivated in the tropics and sub tropics, where it serves as a major food source. Food and Agriculture Organization [1] gave the statistics of world production as 104.6×10^9 kg in 2008. China accounts for 75-80% of worldwide sweet potato production with an annual production of 78.8×10^9 kg followed by Nigeria with about 3.3×10^9 kg. One of the challenges faced by developing countries such as Nigeria is the lack of storage facilities, some million tons of the tubers are destroyed due to improper storage management. In order to proffer solution to this wastage, value addition to these tubers to produce other useful products is imperative.

Sweet potato is a true root that is rich in starch (15-25%). This starch can be hydrolyzed to sugar syrups, which are employed by the food industry to make sweets, drinks, juices and for fermentation into products such as citric acid, gluconic acid and ethanol as well as in paper and textile industry [2,3]. Azhar and Hamdy [4] and Kim and Hamdy [5] have earlier reported acid hydrolysis of sweet potato starch while other studies on enzymatic hydrolysis include the works of Azhar and Hamdy [6], Sawai et al. [7] and Shariffa et al. [8]. None of these reports made use of design of experiment and optimization tool such as Response Surface Methodology (RSM) for their studies. RSM is a comprehensive experimental design and mathematical modeling, through the partial regression fitting of the experimental factors [9]. One of its advantages is that it minimizes the number of experiments needed to be conducted and gives adequate statistical results.

Citric acid is an essential multifunctional organic acid with many uses in the food and pharmaceuticals industries. Global production of citric acid in 2004 was about 1.4 million tonnes [10], which is almost exclusively by fermentation. One important characteristic in the fermentation process is the development of an inexpensive culture medium to achieve maximum product yield. The growth of fungi on sugar-rich agro-materials can be a cost-effective way of producing citric acid [11]. Hence, the utilization of sweet potato starch for this purpose.

This work aimed to determine the optimal conditions for the two-step enzymatic hydrolysis of sweet potato starch using response surface methodology and subsequently using the sweet potato starch hydrolyzate (SPSH) obtained in the process as a feedstock for citric acid production as a way of adding value to sweet potato tubers.

2. METHODOLOGY

2.1 Materials

2.1.1 Sweet potato starch preparation

Sweet potato tubers were obtained from Ogbomoso, Oyo State, Nigeria. The tubers were washed in clean water to remove the adhering dirt; they were peeled manually, and were crushed using hammer mill machine. The crushed pulp was sieved with a sieve of Teflon cloth. The starch obtained was allowed to settle for about 12 h. It was decanted and the starch cake obtained was oven dried. The dried starch was then packed in a container for storage.

2.1.2 Enzymes

For this work, two-step enzymatic hydrolysis method was adopted. In the first step, that is liquefaction, partially purified bacterial alpha amylase (6.4 units/mL) was used while for the second step, that is saccharification, partially purified fungal glucoamylase (789.6 units/mL) was employed. The enzymes were purchased from Federal Institute of Industrial Research Oshodi (Nigeria).

2.2 Methods

2.2.1 Experimental design

Box Behnken Design (BBD) was employed for the design of experiment for the optimization studies. A three-level-three-factor design was applied, which generated 17 experimental runs for each step of the hydrolysis. This included 6 factorial points, 6 axial points and 5 central points to provide information regarding the interior of the experimental region. Response surface methodology (RSM) was used to optimize the process and regression equation analysis was used to evaluate the response surface model. Selected hydrolysis variables considered for both the liquefaction and saccharification steps were temperature (X_1), time (X_2) and pH (X_3). The coded independent variables levels for both liquefaction and saccharification steps are depicted in Table 1 and Table 2, respectively. The independent variables that were used were coded according to Eq. (1):

$$x_i = \frac{X_i - X_0}{\Delta x} \quad i = 1, 2 \dots k \quad (1)$$

where, X_i and x_i are the actual value and codified value, respectively, X_0 is the value of X_i at centre point, and Δx is the step change value. The generalized response surface model for describing the variation in response variable is given as:

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i < j}^k b_{ij} X_i X_j + e \quad (2)$$

where Y is the predicted response by response surface model, i and j are the linear and quadratic coefficients, respectively, b is the regression coefficient, k is the number of factors studied and optimized in the experiment, and e represents the random error.

Table 1. Box-Behnken design for liquefaction step

Variable	Symbol	Coded variable levels		
		-1	0	+1
Temperature (°C)	X ₁	55	60	65
Time (min)	X ₂	55	60	65
pH	X ₃	5.5	6.0	6.5

Table 2. Box-Behnken design for saccharification step

Variable	Symbol	Coded variable levels		
		-1	0	+1
Temperature (°C)	X ₁	40	50	60
Time (min)	X ₂	20	40	60
pH	X ₃	4.0	4.5	5.0

2.2.2 Two-step enzymatic starch hydrolysis

For the hydrolysis studies, the method of Betiku et al. [12] was adopted. The sweet potato flour obtained was made into starch slurry by adding appropriate quantity of water. In order to make 25% (w/v) of slurry, 20 g of flour was weighed into 80 mL of a solution containing 40 ppm Ca²⁺. The pH was adjusted to 6.5 with citrate-phosphate buffer appropriately. The slurried starch was gelatinized by heating the mixture to 97°C for 10 min afterward, 1 % (v/v) of α-amylase was added for liquefaction to take place by using the BBD design in Table 1. Enzyme activities were stopped by heating the mixture to boil. The final mixtures were centrifuged at 10,000 rpm for 10 min and the supernatants were analyzed for reducing sugar. The liquefied starch at the established optimal condition was later subjected to saccharification optimization studies as designed in Table 2. The mixtures were treated as stated above.

2.2.3 Citric acid production

2.2.3.1 Inoculum preparation

Aspergillus niger used in this study was obtained from Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The microorganism was maintained on Potato Dextrose Agar (PDA). Cultures grown on PDA medium in petri dishes were transferred into Duran flask (250 mL) containing 100 mL of sterile distilled water aseptically. The inoculated flasks were shaken continuously on an environment-controlled incubator shaker (New Brunswick Scientific Co., USA) at 200 rpm and 30°C for 1 h before it was used to inoculate the medium for the fermentation.

2.2.3.2 Medium composition for citric acid production

Fermentation medium used for this study composed of carbon source, di-ammonium hydrogen phosphate, (NH₄)₂HPO₄, (DAHP), potassium dihydrogen phosphate, KH₂PO₄, (PDHP), MgSO₄·7H₂O, 0.10 g/L [13]. The carbon source used in this study was sweet potato starch hydrolyzate (SPSH). All media and flasks were sterilized using an autoclave at 121°C for 15 min.

2.2.3.3 Surface fermentation studies for citric acid production

Fifty millilitres of SPSH was measured into 250-mL Duran flasks and the nutrients were added appropriately. The pH of the medium was adjusted using 1 N of HCl and 2 M of NaOH to 6.0. Subsequently, 5% (v/v) of inoculum size was added aseptically to the flask, which was placed on a clean table for surface fermentation.

2.3 Analytical Procedures

2.3.1 Reducing sugar assay

The dinitrosalicylic acid (DNS) method of Miller [14] was used to determine the concentration of SPSH produced, which was expressed as glucose. To 1 mL of the supernatant, 3 mL of the DNS solution was added in the test tube and was boiled for 15 min, cooled and diluted appropriately after which their absorbance were measured at a wavelength of 540 nm using the UV-Visible Spectrophotometer (Libra 21 Model, UK). Dextrose equivalent (DE) was calculated as follows:

$$DE = \left(\frac{\text{Reducing sugar expressed as glucose}}{\text{Sample dry weight}} \right) \times 100 \quad (3)$$

2.3.2 Citric acid analytical technique

Citric acid produced was determined using improved pyridine-acetic anhydride Spectrophotometric method [15]. For the assay, 10 mL of sample was withdrawn from fermentation broth and filtered with Whatman No. 1 filter paper into a flask. Subsequently, 1 mL from the filtrate was mixed thoroughly with 100 mL of distilled water and the resulting solution was used for the citric acid analysis.

2.3.3 Biomass concentration determination

For each sample taken, a pre-weighed, dried filter paper was used to filter the broth, the residue was washed three times with distilled water and dried at a temperature of 120°C for 6 h to a constant weight, it was then allowed to cool and final weight was recorded. The weight of the biomass was determined by subtracting the weight of the filter paper from the filter paper plus the cell mass.

3. RESULTS AND DISCUSSION

3.1 Optimization of Liquefaction Step of Sweet Potato Starch Hydrolysis

Table 3 shows the coded factors considered in this study together with the experimental SPSH concentrations, predicted SPSH concentrations and residual values. Design Expert 8.0.3.1 software was employed to evaluate and determine the coefficients of the full regression model equation and their statistical significance. Table 4 shows the results of test of significance for every regression coefficient. The results showed that the p-values of the model terms were significant, i.e. $P < 0.05$. In this case, the three linear terms (X_1 , X_2 , X_3), three cross-products (X_1X_2 , X_1X_3 , X_2X_3) and the three quadratic terms (X_1^2 , X_2^2 , X_3^2) were all markedly significant model terms at 95% confidence level. The analysis of variance of the

regression equation model is presented in Table 5. The model F-value of 2401.41 and $P < 0.0001$ implied the model was significant. The data obtained fitted best to a quadratic model. It exhibited high coefficient of determination (R^2), which should be at least 0.80 for the good fit of a model [16]. The R^2 and R^2 (adjusted) obtained for the model were 0.997 and 0.993, respectively, which demonstrated that the model proved suitable for the adequate representation of the actual relationship among the selected factors.

$$Y = 129.75 + 5.36X_1 + 0.26X_2 + 2.00X_3 - 0.31X_1X_2 + 7.38X_1X_3 - 1.95X_2X_3 - 40.63X_1^2 + 22.64X_2^2 + 15.59X_3^2 \quad (4)$$

Table 3. Liquefaction data for experimental SPSH concentration, predicted SPSH concentration and residual values

Std run	X_1	X_2	X_3	Experimental concentration (g/L)	Predicted Concentration (g/L)	Residual Value
1	-1	-1	0	105.48	105.82	-0.34
2	1	-1	0	116.82	117.16	-0.34
3	-1	1	0	107.47	106.96	0.51
4	1	0	-1	117.57	117.07	0.51
5	-1	0	-1	105.02	104.72	0.30
6	1	0	1	101.00	100.70	0.30
7	-1	0	1	93.5	93.97	-0.47
8	1	0	1	118.98	119.44	-0.47
9	0	1	-1	163.89	163.77	0.12
10	0	-1	-1	167.48	168.20	-0.72
11	0	-1	1	172.22	171.67	0.55
12	0	1	1	168.00	168.29	-0.29
13	0	0	0	129.70	129.75	-0.05
14	0	0	0	129.00	129.75	-0.05
15	0	0	0	148.00	147.34	0.66
16	0	0	0	129.98	129.75	0.23
17	0	0	0	129.98	129.75	0.23

Table 4. Test of significance for every regression coefficient for the liquefaction step

Source	Sum of Square	df	Mean Square	F-value	P-value
X_1	230.05	1	230.05	416.94	<0.0001
X_2	0.56	1	0.56	1.15	0.3185
X_3	35.08	1	35.08	72.69	<0.0001
$X_1 X_2$	0.38	1	0.38	0.80	0.4017
$X_1 X_3$	217.56	1	217.56	450.56	<0.0001
$X_2 X_3$	15.25	1	15.25	31.60	0.0008
X_1^2	6918.98	1	6918.98	14338.45	<0.0001
X_2^2	2147.63	1	2147.63	445.61	<0.0001
X_3^2	1019.01	1	1019.01	2111.73	<0.0001

Table 5. Analysis of variance (ANOVA) of regression equation for the liquefaction step

Source	Sum of squares	df	Mean Square	F-value	P-value
Model	10455.21	9	1161.69	2401.41	<0.0001
Residual	3.38	7	0.48		
Lack of Fit	2.74	4	0.68	3.20	0.1835
Pure Error	0.64	3	0.21		
Cor Total	10458.59	16			
			$R^2 = 0.997$		$R^2 \text{ (adj)} = 0.993$

Also, these values confirmed that the regression was statistically significant; only 0.3% of total variations were not explained by this regression model. The lack-of-fit term greater than 0.05 was not significant, which showed that the model was significant for the response. Therefore, it could be used in theoretical prediction of liquefaction of sweet potato starch. The final equation in terms of coded factors for the Box-Behnken response surface quadratic model is expressed in Eq. (4). The low values of standard error observed in the intercept and all the model terms showed that the regression model fits the data well, and the prediction was good (Table 6).

Table 6. Regression coefficients and significance of response surface quadratic for the liquefaction step

Factor	Coefficient estimate	df	Standard error	95%CI low	95%CI high	VIF
Intercept	129.75	1	0.34	128.95	130.55	-
X_1	5.36	1	0.25	4.78	5.94	1.00
X_2	0.26	1	0.25	-0.38	0.84	1.02
X_3	2.00	1	0.23	1.44	7.55	1.00
X_1X_2	-0.31	1	0.35	-1.13	0.51	1.00
X_1X_3	7.38	1	0.35	6.55	8.2	1.00
X_2X_3	-1.95	1	0.35	-2.77	-1.13	1.00
X_1^2	-4.63	1	0.34	41.44	39.85	1.01
X_2^2	22.64	1	0.34	21.84	23.44	1.01
X_3^2	15.59	1	0.34	14.79	16.4	1.01

The three dimensional (3-D) surface graph provides a kind of visual method to observe responsive value and to test the parameter level of interaction. Fig. 1(A-C) shows the response surface plots for the liquefaction step in the sweet potato starch hydrolysis.

The curvatures nature of 3D surfaces indicated the mutual interaction of the hydrolysis time with hydrolysis temperature, pH with hydrolysis temperature and pH with hydrolysis time. The optimal SPSH concentration for liquefaction step was 172.22 g/L established at 60°C, 60 min and pH of 6.5. The predicted SPSH concentration under the above condition was $Y = 172.232\text{g/L}$. To verify the prediction of the model, the optimal condition was applied to three independent replicates and the average SPSH concentration obtained was 172.00 g/L (DE of 68.8), which is well within the estimated value of the model equation.

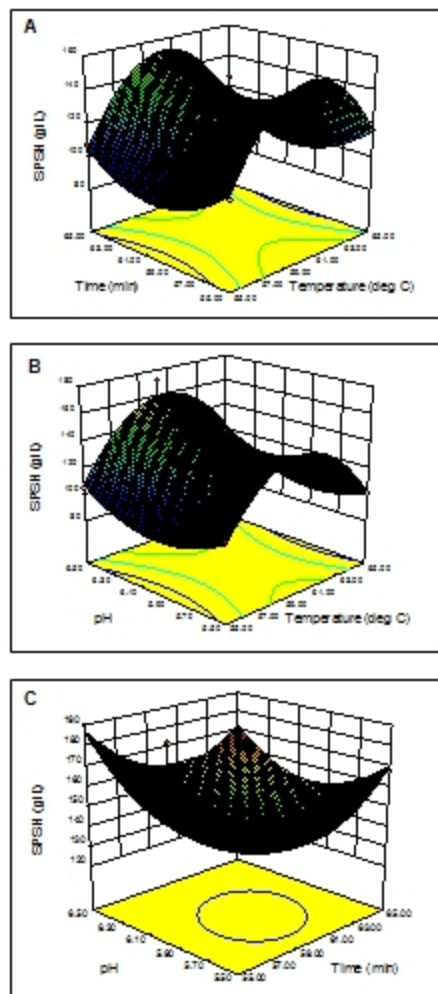


Fig. 1. Surface plots for liquefaction of sweet potato starch

3.2 Optimization of Saccharification Step of Sweet Potato Starch Hydrolysis

Saccharification step was introduced into the study due to the significant amount of unhydrolyzed starch left after the liquefaction step. Results of the saccharification step are shown in Table 7, which contained the coded factors together with experimental SPSH concentrations and predicted SPSH concentrations as well as the residual values. Table 8 shows the results of test of significance for every regression coefficient. The results showed that all the p-values of the model terms were significant i.e $P < 0.05$. The three linear term (X_1, X_2, X_3), three cross products (X_1X_2, X_1X_3, X_2X_3) and the three quadratic terms (X_1^2, X_2^2 and X_3^2) were all remarkably significant model terms at 95% confidence level. Table 9 depicts the analysis of variance of the regression equation model. The model F-value of 12357.00 implied the model was significant. As observed in the liquefaction step, the data obtained fitted best to a quadratic model. The R^2 and R^2 (adjusted) obtained for the model were 0.997 and 0.994, respectively, which established that the model proved suitable for the

adequate representation of the actual relationship among the selected factors. Also, these values indicated that this regression was statistically significant; only 0.3% of total variations were not explained by this regression model. The lack-of-fit term greater than 0.05 was not significant, which revealed that the model was significant for the response. Therefore, it could be used in theoretical prediction of saccharification of sweet potato starch.

Table 7. Saccharification data for experimental SPSH concentrations, predicted SPSH concentrations and residual values

Std run	X ₁	X ₂	X ₃	Experimental concentration (g/L)	Predicted concentration (g/L)	Residual values
1	-1	-1	0	164.96	165.82	-0.86
2	1	-1	0	157.00	155.93	1.67
3	-1	1	0	168.34	167.41	-1.07
4	1	1	0	207.30	206.44	0.86
5	-1	0	-1	205.71	203.51	2.20
6	1	0	-1	209.73	209.46	0.27
7	-1	0	1	215.43	215.69	-0.26
8	1	0	1	234.67	236.57	-2.20
9	0	-1	-1	181.00	182.34	-1.34
10	0	1	-1	215.40	216.53	-1.13
11	0	-1	1	210.40	209.77	1.13
12	0	1	1	230.52	229.18	1.34
13	0	0	0	237.00	237.82	-0.82
14	0	0	0	237.10	237.82	-0.72
15	0	0	0	237.10	237.82	-0.82
16	0	0	0	237.00	237.82	-0.82
17	0	0	0	241.02	237.82	3.20

Table 8. Test of significance for every regression coefficient for saccharification step

Source	Sum of squares	df	Mean square	F-value	P-value
X ₁	368.02	1	368.02	79.25	<0.0001
X ₂	1463.40	1	1463.40	315.15	<0.0001
X ₃	783.68	1	783.68	168.77	<0.0001
X ₁ X ₂	550.37	1	550.37	118.52	<0.0001
X ₁ X ₃	57.91	1	57.91	12.57	0.0096
X ₂ X ₃	50.98	1	50.98	10.98	0.0129
X ₁ ²	3344.70	1	3344.70	720.29	<0.0001
X ₂ ²	5228.73	1	5228.73	1126.03	<0.0001
X ₃ ²	191.59	1	191.59	41.26	0.0004

The final equation in terms of coded factors for the response surface quadratic model is expressed in Eq. (5):

$$Y = 237.82 + 6.78X_1 + 13.53X_2 + 9.90X_3 + 11.73X_1X_2 + 3.81X_1X_3 - 3.57X_2X_3 - 28.18X_1^2 - 35.24X_2^2 + 6.75X_3^2 \quad (5)$$

The low values of standard error observed in the intercept and all the model terms showed that the regression model fits the data well, and the prediction was good (Table 10).

Table 9. Analysis of variance (ANOVA) of regression equation for saccharification step

Source	Sum of squares	df	Mean square	F-value	P-value
Model	12357.00	9	1373.00	295.68	<0.0001
Residual	32.50	7	4.64		
Lack of Fit	19.73	3	6.58	2.06	0.2483
Pure Error	12.78	4	3.19		
Cor Total	12389.59	16			
			$R^2 = 0.997$	R^2 (adj) = 0.994	

Table 10. Regression coefficients and significance of response surface quadratic for saccharification step

Factor	Coefficient estimate	df	Standard error	95%CI low	95%CI high	VIF
Intercept	237.82	1	0.96	235.55	240.10	-
X_1	6.78	1	0.76	4.98	8.58	1.00
X_2	13.53	1	0.76	11.72	15.33	1.02
X_3	9.90	1	0.76	8.10	11.70	1.00
X_1X_2	11.73	1	1.08	9.18	14.28	1.00
X_1X_3	3.81	1	1.08	1.26	6.35	1.00
X_2X_3	-3.57	1	1.08	-6.12	-1.02	1.00
X_1^2	-28.18	1	1.05	-30.67	-25.70	1.01
X_2^2	-35.24	1	1.05	-37.72	-32.72	1.01
X_3^2	6.75	1	1.05	4.26	4.26	1.01

Fig. 2(A-C) represents the response surface plot for the saccharification step in the sweet potato starch hydrolysis.

The curvatures nature of 3-D surfaces indicated the mutual interaction of pH with hydrolysis temperature, hydrolysis time with pH and hydrolysis time with hydrolysis temperature. The optimal condition values of the independent variables selected for the saccharification step were also obtained by solving the regression equation (Eq. 5) using the Design-Expert software. The values obtained were temperature 52°C, time 44 min and pH of 4.5. The predicted SPSH concentration under the above set of values was $Y = 241.92$ g/L. To verify the prediction of the model, the optimal condition was applied to three independent replicates and the average SPSH concentration obtained was 242.02 g/L (DE of 96.8), which is well within the predicted value of the model equation.

The results of this work have shown that response surface methodology could be used to optimize the two-step enzymatic hydrolysis of sweet potato starch.

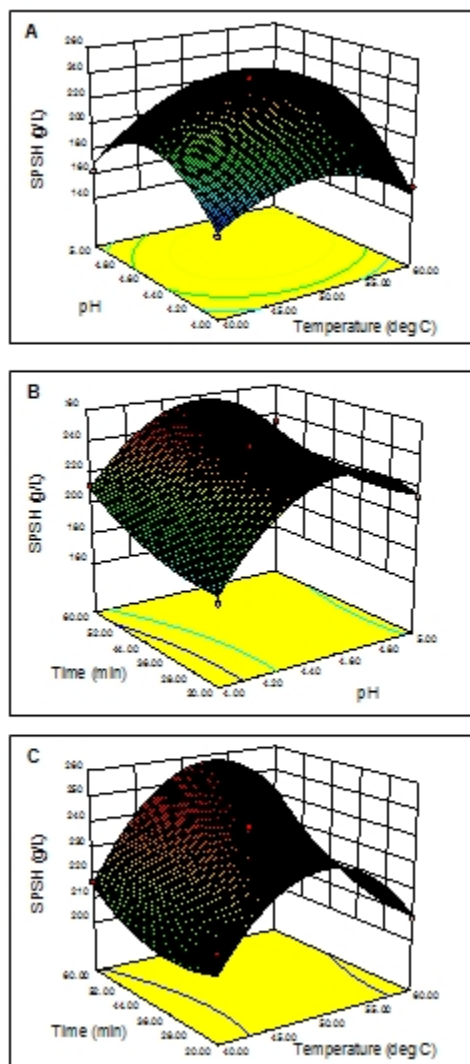


Fig. 2. Surface plots for saccharification of liquefied sweet potato starch

3.3 Fermentation of SPSH for Citric Acid Production

The study investigated the possible use of sweet potato starch hydrolyzate (SPSH) as the sole carbon source for the production of citric acid using *Aspergillus niger* under surface fermentation. Fig. 3 shows the profile of citric acid concentration, SPSH concentration and biomass concentration against time for surface fermentation. The results showed that *A. niger* was able to metabolize the SPSH without difficulty. The microorganism was able to convert 93% of the SPSH within 15 days. The citric acid formation increased from 1st day till the 8th day, after which there was decline in the concentration of the acid. This may be attributed to the depletion of the SPSH, reduction in nutrient of the medium, formation of other metabolites and decrease in the pH of the medium, which do not favour citric acid synthesis.

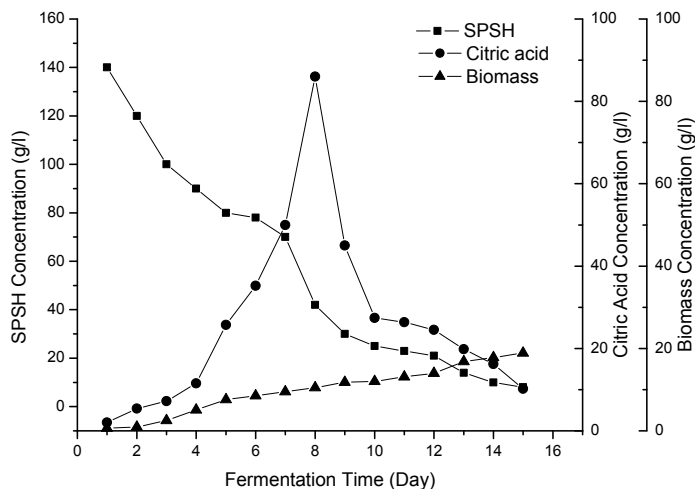


Fig. 3. Plots of SPSH, citric acid and biomass concentrations against fermentation time

The highest concentration of citric acid obtained on the 8th day was 86 g/L. It was also observed that as the fungus consumes the nutrient in the medium, there was a progressive decrease in SPSH from its initial concentration of 150 g/L on the 1st day to 8 g/L on the 15th day. Moreover, the mycelium cell grew well in the medium, as the SPSH concentration reduced, the biomass concentration increased. The highest biomass concentration obtained was 18.9 g/L on the 15th day. Yuguo et al. [17] achieved 106 g/L citric acid from mash of dried sweet potato in 65 h using external-loop air lift bioreactor. In another study, Anwar et al. [18] carried out surface fermentation of hydrolyzed sweet potato starch using *A. niger* and reported maximum citric acid production of 23.87 g/L in 264 h. Dhillon et al. [11] achieved 18.34 g/L of citric acid in 120 h using brewery spent liquid supplemented with apple pomace ultrafiltration sludge in a submerged fermentation.

4. CONCLUSIONS

Response surface methodology was successfully applied in the two-step enzymatic hydrolysis of sweet potato starch. The maximum SPSH concentration obtained for the liquefaction step was 172.22 g/L (DE = 68.9) at temperature of 60°C, time 60 min, and pH of 6.5. For the saccharification step, the SPSH concentration increased to 241.02 g/L (DE = 96.8) at time 44 min, temperature 52°C and pH of 4.5. The SPSH obtained was further used as the feedstock for *Aspergillus niger*, which was subsequently converted to citric acid with a maximum production of 86 g/L.

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COMPETING INTERESTS

Authors declare no conflict of interest.

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